REMARKS

Status of the Claims

Claims 1-21, 23-41 and 125 are under examination. Claims 1, 4-12, 17, 18, 35, 36, 38, 40, 41 and withdrawn claims 72 and 86 are amended herein.

For convenience, reference to support in the Specification refers to the published application (publication No. 20040219203). Claim 1 is amended to replace "binding molecules" with "antibodies or antigen-binding fragments thereof." The amendment is supported in the published Specification at least at Paragraphs [0016]-[0019]. Dependent claims 4-12, 17, 18, 35, 36, 38, 40 and 41 and withdrawn claim 86 are amended to be consistent with the amendment to claim 1. Claim 21 is also amended to replace "binding molecule" with "antibody or antigen-binding fragment thereof."

Claims 38, 40, 41 and 72 are amended to correct spelling or grammatical errors. Claim 38 is also amended to delete the phrase "part or all" of the light and heavy chains of $F(ab')_2$, Fab, scFv,or Fv Applicants respectfully submit that no new matter is added by amendment.

Priority Date

The Action asserts that the effective priority date of the instant claims is the June 17, 2003, filing date of 60/478,830. The Action further asserts that, "The prior applications 10/314,330, 10/350,096, 10/377,122 fail to provide a written description of the instant claims composition comprising nanoparticles or any submicron particles conjugated to a CD74 binding molecule and in combination with one or more effectors."

Applicants respectfully traverse. The instant application is a continuation-in-part of USSN 10/314,330, which was a continuation of USSN 09/965,796, which was a continuation of 09/307,816, filed May 10, 1999. For the reasons stated below, Applicants assert that the instant claims are entitled to the May 10, 1999 priority date of USSN 09/307,816.

For convenience, the following text refers to U.S. Patent No. 6,306,393 (the '393 patent), which issued from USSN 09/307,816. However, Applicants note that USSN 09/965,796 and USSN 10/314,330 were continuations of USSN 09/307,816 and contain similar or identical text.

Applicants initially note that use of anti-CD74 antibodies conjugated to therapeutic agents was explicitly contemplated and claimed in the '393 patent (see, e.g., claim 33). The '393 patent describes in great detail the, "Coupling of Antibodies, **Immunoconjugates** and Fusion Proteins to Lipid Emulsions." ['393 patent, Col. 11, line 20 to Col. 12, line 31 (emphasis added)]

> Long-circulating **sub-micron lipid emulsions**, stabilized with poly(ethylene glycol)-modified phosphatidylethanolamine (PEG-PE), can be used as drug carriers for the anti-CD22 and anti-CD19 antibody components, **immunoconjugates**, and fusion proteins of the present invention. The emulsions are composed of two major parts: an oil core, e.g., triglyceride, stabilized by emulsifiers, e.g., phospholipids. The poor emulsifying properties of phospholipids can be enhanced by adding a biocompatible co-emulsifier such as polysorbate 80. In a preferred embodiment, the anti-CD22 and anti-CD19 antibody components, immunoconjugates and fusion proteins are conjugated to the surface of the lipid emulsion globules with a poly(ethylene glycol)-based, heterobifunctional coupling agent, poly(ethylene glycol)-vinylsulfone-N-hydroxy-succinimnidyl ester (NHS-PEG-VS).
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> The **submicron lipid emulsion** is prepared and characterized as described. Lundberg, J. Pharm. Sci., 83:72 (1993); Lundberg et al., Int. J. Pharm., 134:119 (1996). The basic composition of the lipid emulsion is triolein:DPPC:polysorbate 80, 2:1:0.4 (w/w). When indicated, PEG-DPPE is added into the lipid mixture at an amount of 2-8 mol % calculated on DPPC.
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> The coupling procedure starts with the reaction of the NHS ester group of NHS-PEG-VS with the amino group of distearoyl phosphatidyl-ethanolamine (DSPE). Twenty-five .mu.mol of NHS-PEG-VS are reacted with 23 .mu.mol of DSPE and 50 .mu.mol triethylamine in 1 ml of chloroform for 6 hours at 40.degree. C. to produce a poly(ethylene glycol) derivative of phosphatidyl-ethanolamine with a vinylsulfone group at the distal terminus of the poly(ethylene glycol) chain (DSPE-PEG-VS). For antibody conjugation, DSPE-PEG-VS is included in the lipid emulsion at 2 mol % of DPPC. The components are dispersed into vials from stock solutions at -20.degree. C., the solvent is evaporated to dryness under reduced pressure. Phosphate-buffered saline (PBS) is added, the mixture is heated to 50.degree. C., vortexed for 30 seconds and sonicated with a MSE probe sonicator for 1 minute. Emulsions can be stored at 4.degree. C., and preferably are used for conjugation within 24 hours.
>
> Coupling of anti-CD22 or anti-CD19 antibodies to emulsion globules is performed via a reaction between the vinylsulfone group at the distal PEG terminus on the surface of the globules and free thiol groups on the antibody. Vinylsulfone is an attractive derivative for selective coupling to thiol groups. At approximately neutral pH, VS will couple with a half life of 15-20 minutes to proteins containing thiol groups. The reactivity of VS is slightly less than that of maleimide, but the VS group is more stable in water and a stable linkage is produced from reaction with thiol groups.

Before conjugation, the antibody is reduced by 50 mM 2-mercaptoethanol for 10 minutes at 4.degree. C. in 0.2 M Tris buffer (pH 8.7). The reduced antibody is separated from excess 2-mercaptoethanol with a Sephadex G-25 spin column, equilibrated in 50 mM sodium acetate buffered 0.9% saline (pH 5.3). The product is assayed for protein concentration by measuring its absorbance at 280 nm (and assuming that a 1 mg/ml antibody solution of 1.4) or by quantitation of .sup.125 I-labeled antibody. Thiol groups are determined with Aldrithiol.TM. following the change in absorbance at 343 nm and with cystein as standard.

The coupling reaction is performed in HEPES-buffered saline (pH 7.4) overnight at ambient temperature under argon. Excess vinylsulfone groups are quenched with 2 mM 2-mercaptoethanol for 30 minutes, excess 2-mercaptoethanol and antibody are removed by gel chromatography on a Sepharose CL-48 column. The imnunoconjugates are collected near the void volume of the column, sterilized by passage through a 0.45 .mu.m sterile filter, and stored at 4.degree. C.

Coupling efficiency is calculated using .sup.125 I-labeled antibody. Recovery of emulsions is estimated from measurements of [.sup.14 C]DPPC in parallel experiments. The conjugation of reduced LL2 to the VS group of surface-grafted DSPE-PEG-VS is very reproducible with a typical efficiency of near 85%.

Applicants note that the cited passage explicitly refers to conjugation of antibodies or **immunoconjugates** to sub-micron lipid emulsions. Further, issued claim 33 of the '393 patent explicitly claims methods of use of **anti-CD74 immunoconjugate**. An "immunoconjugate" is defined in the '393 patent at Col. 4, lines 30-31: "An immunoconjugate is a conjugate of an antibody component with a therapeutic agent."

Thus, the '393 patent expressly claims use of anti-CD74 antibodies conjugated to therapeutic agents (i.e., immunoconjugates) and contains a detailed description of the conjugation of such immunoconjugates to sub-micron lipid emulsions, as claimed in instant Claim 1. The 5/10/99 priority document contains ample 35 U.S.C. §112 support for the presently pending claims and the instant application is entitled to the claimed priority date of USSN 09/307,816. Reconsideration and award of the claimed priority dates are respectfully requested.

Rejection of Claims Under 35 U.S.C. 112, 1st Paragraph

Claims 1-7, 9-21, 23-35, 38, 39 and 125 are rejected under 35 U.S.C. 112 1st paragraph for lack of written description. The Action asserts that, "The instant specification

provides a written description of only antibodies which bind to CD74." Although Applicants traverse the assertion, in the interests of advancing prosecution the claims are amended herein to replace "binding molecules" with "antibodies or antigen-binding fragments thereof." Paragraph [0047] of the published Specification states that an "antibody" refers to a full-length immunoglobulin molecule or "an immunologically active (i.e., specifically binding) portion of an immunoglobulin molecule, like an antibody fragment."

Claim 21 is also rejected under 35 U.S.C. 112 1st paragraph. The Action asserts that, "Neither the specification nor the claims provide a target or a structure fro [sic] the binding molecule; neither the specification not [sic] the claims provides a function for the 'oligonucleotide'."

Although Applicants respectfully traverse the assertion, in the interest of advancing prosecution the term "binding molecule" is replaced with "antibody or fragment thereof." Applicants submit that the Specification and claims provide both targets and structures for the effector antibody or fragment. For example, original claim 11 disclosed binding molecules (such as antibodies or fragments) "which specifically bind to one or more antigens selected from the group consisting of CD4, CD5, CD8, CD14, CD15, CD19, CD20, CD21, CD22, CD23, CD25, CD30, CD33, CD37, CD38, CD40, CD40L, CD46, CD52, CD54, CD80, CD126, B7, MUC1, MUC2, MUC3, MUC4, Ia, HM1.24, tensascin, VEGF, EGFR, CEA, CSAp, ILGF, placental growth factor, carbonic anhydrase IX, IL-6 and combinations thereof." The Specification at paragraph [0009] further discloses, "antibodies or antigen-binding fragments thereof that bind to CD19, CD20, CD22, CD30, CD33, CD52, CD80, HLA-DR, MUC1, TAC, IL-6, tensascin, VEGF, placental growth factor, carbonic anhydrase IX, and mixtures thereof." Antibodies and fragments thereof that bound to those target antigens were well known in the art as of the instant priority date.

The Specification and claims also provide explicit support for the function and structure of oligonucleotide effectors. For example, at Paragraph [0012] the Specification states that an "oligonucleotide molecule" may be "e.g., an antisense molecule or a gene." "Antisense molecules may include antisense molecules that correspond to bcl-2 or p53." Antisense molecules are well known in the art as oligonucleotides that inhibit the function of particular genes, by binding to mRNA products of those genes. As the sequences of bcl-2 and p53 are also well known in the art, Applicants submit that both the structure and function

of "antisense molecules that correspond to bcl-2 or p53" would be clear to the skilled artisan, reading the instant Specification in light of general knowledge in the art.

Claims 38 and 39 are also rejected under 35 U.S.C. 112 1st paragraph. The Action asserts that, "formation of an intact antigen-binding site generally requires the association of the complete heavy and light chain variable regions of a given antibody." Applicants respectfully traverse, but in the interest of advancing prosecution have deleted the recitation in claim 38 to "part of" the light and heavy chains. Applicants further submit that the published Specification describes in great detail how the skilled artisan could make and use a fusion protein within the scope of the instant claims, for example at Paragraphs [0021], [0057], [0059], [0064], [0067], [0080], [0081], [0084], [0101], [102] and [0104]. Applicants respectfully submit that the skilled artisan, reading the specification in light of general knowledge in the art, would be able to make and use the claimed subject matter without undue experimentation.

Claims 11, 12, 21, 30-34, 39 and 41 are also rejected under 35 U.S.C. 112 1st paragraph. The Action states that the Specification is enabling for effectors that are drugs, toxins, radioisotopes or a photodynamic agent and CD74 binding antibodies, but asserts that it "does not reasonably provide enablement for antibodies which bind to CD74 which are multispecific and inclusion of further binding molecules which bind to antigens which are not known to be related to the B cell dyscrasias or hematopoietic cells expressing MHCII, such as CD4, CD5, CD8, CD40L, etc. and effectors which are immunomodulators, enzymes, hormones, or antiangiogenic molecules." The Action further asserts that, "The specification has not provided any objective evidence that non-hematopoietic solid tumors express CD74. The prior art teaches that the LL1 antibody is a CD74 binding antibody and that the LL1 antibody is specific for B lymphocytes, monocytes and histiocytes because it binds to the Ii (invariant chain) of the MHC II antigen, and lacks cross reaction with solid tumors, multiple myelomas and myelogenous leukemia." (citations omitted)

Applicants respectfully traverse the assertion that CD74 positive cells are not found in solid tumors. Attached are abstracts reporting the presence of CD74 positive cells in solid tumors of breast (Tupitsyn et al., 1994), breast carcinoma cells (Lu et al., 1994), lung tumors (Ioachim et al., 1996) and renal cell carcinoma (Saito et al., 1993).

Applicants submit that multispecific antibodies and uses thereof were well known in the art as of the instant priority date and are described in detail in the published Specification, for example at Paragraphs [0078]-[0086]. As stated in Paragraph [0080],

> [A]nother preferred antibody fusion protein contains one or more Fvs, or Fab's of the mAbs or fragments thereof of a humanized, chimeric, human or murine anti-CD74 mAb or fragment thereof as described herein, and one or more Fvs or Fab's from antibodies specific for another antigen that is specific for a tumor cell marker that is not a CD74 antigen. For example, the non-CD74 antigen may be expressed by the CD74-expressing cells and may include a tumor marker selected from a B-cell lineage antigen, (e.g., CD19, CD20, or CD22 for the treatment of B-cell malignancies). The non-CD74 antigen may also be expressed on other CD74 positive cells that cause other types of malignancies, such as S100 in melanoma, etc. Further, the tumor cell marker may be a non-B-cell lineage antigen selected from the group consisting of HLA-DR, CD30, CD33, CD52 MUC1 and TAC.

Bispecific antibodies and methods for their production and use were well known in the art. For example, U.S. 5,851,527, teaches bispecific antibodies or fragments having a first binding site specific for a target antigen and a second binding site specific for an epitope on an enzyme. U.S. 6,083,477 taught bispecific antibodies with a first specificity for a malignant cell marker and a second specificity for IL-15α. Under MPEP 2164.01, "The test of enablement is whether one reasonably skilled in the art could make or use the invention from the disclosures in the patent coupled with information known in the art without undue experimentation." United States v. Telectronics, Inc., 857 F.2d 778, 785, 8 USPQ2d 1217, 1223 (Fed. Cir. 1988). "A patent need not teach, and preferably omits, what is well known in the art." In re Buchner, 929 F.2d 660, 661, 18 USPQ2d 1331, 1332 (Fed. Cir. 1991); Hybritech, Inc. v. Monoclonal Antibodies , Inc ., 802 F.2d 1367, 1384, 231 USPQ 81, 94 (Fed. Cir. 1986), cert. denied, 480 U.S. 947 (1987); and Lindemann Maschinenfabrik GMBH v. American Hoist & Derrick Co., 730 F.2d 1452, 1463, 221 USPQ 481, 489 (Fed. Cir. 1984).

Applicants submit that the Specification is fully enabling for use of therapeutic immunomodulators, enzyme, hormones and antiangiogenic agents, as described in the published Specification for example at Paragraphs [0015], [0057], [0097], [0102] and [0125]. Use of antibody conjugates comprising therapeutic immunomodulators, enzyme, hormones

and antiangiogenic agents was also well known in the art, for example U.S. Patent No. 5,482,698; 5,686,578; 5,697,902; 5,716,595 and 5,851,527.

Reconsideration and withdrawal of the rejection are requested.

Rejection of Claims Under 35 U.S.C. 102

Claims 1, 2, 6, 8, 20, 21, 24, 25, 26, 28, 29, 35 and 125 are rejected under 35 U.S.C. 102(b) as anticipated by Juweid et al. (Nucl. Med. Com. 1997 18:142-48, "Juweid"). The Action asserts that, "Juweid et al disclose a 99Tcm-LL1 antibody conjugate in a sulfur colloid as a carrier (abstract) which meets the limitations of claims 1 requiring a micelle, claim 2 requiring a emulsion, claim 6 wherein the anti-CD74 antibody is conjugated to one or more micelles, and claim 8 requiring a fragment of LL1."

Applicants respectfully traverse the assertion. For convenience, the abstract of Juweid is reproduced below:

Nucl Med Commun. 1997 Feb;18(2):142-8.

99Tcm-LL1: a potential new bone marrow imaging agent.

Juweid M, Dunn RM, Sharkey RM, Rubin AD, Hansen HJ, Goldenberg DM.

Garden State Cancer Center, Center for Molecular Medicine and Immunology, Belleville, NJ 07103, USA.

LL1, a monoclonal antibody (MAb) to HLA Class-II-like antigen (li determinant) on the surface of B-lymphocytes, monocytes and histiocytes, was evaluated as an agent for bone marrow imaging. Six patients with diverse diseases (non-Hodgkin's lymphoma, n = 2; multiple myeloma, n = 1; polycythaemia vera, n = 1; lung cancer, n = 1; breast cancer, n = 1) were given low protein doses (< 1 mg) of 99Tcm (30 mCi) labelled Fab' of LL1. 99Tcm-sulphur colloid (SC) imaging was performed in three patients for comparison. Both planar and single photon emission tomographic images were acquired using Sopha gamma cameras. As early as 2 h post-MAb injection, excellent bone marrow images were achieved in all patients, demonstrating both normal or hyperproliferative marrow, as well as 'cold' bone marrow abnormalities such as radiation defects or cancer metastases. Similar to SC, relatively high uptake of LL1 was found in the liver and spleen. However, the bone marrow-to-liver and -spleen uptake ratios were approximately 19-fold higher (0.75 vs 0.04) and 6-fold higher (1.23 vs 0.22), respectively, with LL1 than with SC. The higher bone marrow uptake allowed clearly superior visualization of the thoracic spine when compared to SC. The mean T1/2 of blood and whole-body clearance were 0.4 and 66 h, respectively. The highest radiation absorbed doses (in cGy mCi-1) were observed in the spleen (0.47 +/- 0.24), kidneys (0.25 +/- 0.09) and liver (0.14 +/- 0.04). The bone marrow dose was only 0.05 +/- 0.02 cGy mCi-1. These results indicate that bone

> marrow imaging with 99Tcm-LL1 is feasible, and that LL1 may be a suitable alternative to SC because of better visualization of the lower thoracic spine. Potential applications include the improved detection of bone marrow metastases of solid tumours and the assessment of haematological disorders. (Emphases added)

Applicants note that the reference to 99Tcm-sulphur colloid (SC) imaging is in a separate sentence from the reference to LL1 antibody administration. The remainder of the abstract makes clear that the 99Tcm-sulphur colloid was a separate treatment that was used as a control or comparison for 99Tcm-LL1 treatment. For example, "Similar to SC, relatively high uptake of LL1 was found in the liver and spleen." And, "the bone marrow-to-liver and -spleen uptake ratios were approximately 19-fold higher (0.75 vs 0.04) and 6-fold higher (1.23 vs 0.22), respectively, with LL1 than with SC." The latter sentence clearly establishes that LL1 antibody was administered separately from sulphur colloid (SC) administration, since if the LL1 antibody were conjugated to the sulphur colloid then the bone marrow-to-liver and – spleen uptake ratios would by necessity be the same for LL1 and SC distribution, not differing by an order of magnitude. Finally, the abstract states that, "LL1 may be a suitable alternative to SC" (emphasis added). I.e., the two alternatives are used separately, not together. The statement is made that LL1 may be an alternative to sulphur colloid, not as an adjunct or conjugate to sulphur colloid, but instead of the colloid.

There is no evidence of record that Juweid contained any teaching, disclosure or even mere suggestion to make the claimed combination of one or more anti-CD74 antibodies or fragments thereof conjugated to one or more lipids, polymeric carriers, micelles or nanoparticles in a composition with one or more effectors. As this element of the instant claimed subject matter is missing from the cited prior art, rejection under 35 U.S.C. 102 is improper. Reconsideration and withdrawal of the rejection are respectfully requested.

Rejection of Claims Under 35 U.S.C. 103

Claims 1-10, 13-21, 27, 35 and 125 are rejected under 35 U.S.C. 103(a) as unpatentable over Pawlak-Byczkowska et al. (Canc. Res. 1989, 49:4568-77, "Pawlak-Byczkowska") as evidenced by Juweid in view of Lundberg et al. (J. Pharm. Pharmacol. 1999, 51:1099-1105, "Lundberg") and Hansen et al. (Biochem. J. 1996, 320:293-300, "Hansen"). The Action states that Pawlak-Byczkowsaka teach the EPB-1 antibody

(identified by Juweid to be LL1) and suggested EPB-1 as a candidate for radioimmunodetection and radioimmunotherapy of B cell neoplasms. The Action acknowledges that "Pawlak-Byczkowsaka et al do not teach the specific composition comprising a LL1 conjugate and one or more effectors." Thus, Pawlak-Byczkowsaka clearly could not have taught the claimed combination of one or more anti-CD74 antibodies or fragments thereof conjugated to one or more lipids, polymeric carriers, micelles or nanoparticles in a composition with one or more effectors, and cannot make up for the deficiencies of Juweid described in the above 102 discussion (incorporated herein by reference).

The Action asserts that Lundberg et al teach conjugation of the **LL2 antibody** with a long-circulating drug carrier lipid emulsion and that "Lundberg et al tech [sic] that submicron lipid emulsions have hydrophobic cores which can solubilize considerable amounts of lipophilic drugs (page 1099, second column, lines 12-14), which fulfills the specific embodiment of claim 7 requiring a nanoparticle.

Applicants note that the section of USSN 09/307,816 cited above in the discussion of the priority date also described antibody conjugation to submicron lipid emulsions which can solubilize considerable amounts of lipophilic drugs. If, as asserted, such a description "fulfills the specific embodiment of claim 7 requiring a nanoparticle," then the instant application is entitled to the claimed May 10, 1999 priority date of the USSN 09/307,816 application. Further, if the present claims to nanoparticle conjugates are entitled to the 5/10/99 priority date, then Lundberg is not a prior art reference, as on its face the Lundberg publication was published in October, 1999. [See PubMed abstract below, showing an October 1999 listed publication date.]

J Pharm Pharmacol. 1999 Oct;51(10):1099-105.

Conjugation of an anti-B-cell lymphoma monoclonal antibody, LL2, to long-circulating drug-carrier lipid emulsions.

Lundberg BB, **Griffiths G**, **Hansen HJ**.

Department of Biochemistry and Pharmacy, Abo Akademi University, BioCity, Finland.

Long-circulating submicron lipid emulsions, stabilized with poly(ethylene

glycol)-modified phosphatidylethanolamine (PEG-PE), are promising drug carriers with substantial capacity for solubilization of lipophilic anticancer agents. This study describes the conjugation of the anti-B-cell lymphoma monoclonal antibody LL2 to the surface of lipid-emulsion globules by use of a novel poly(ethylene glycol)-based heterobifunctional coupling agent. The efficiency of coupling of LL2 to the lipid emulsion was 85% (approx.) and essentially independent of the LL2/emulsion particle ratio and amount of surface-bound PEG-PE. Results from sucrose-gradient centrifugation and Sepharose CL-4B gel filtration indicated stable binding of the antibody to the emulsion. The immunoreactivity of the emulsion-LL2 conjugates was tested with alkaline phosphatase-conjugated LL2 against a monoclonal anti-idiotype antibody, WN. The binding of the conjugates to WN increased with increasing surface density of LL2 up to 40 monoclonal antibodies/emulsion particle, and exceeded that for the free monoclonal antibody (approx. 20 molecules/particle). Results from competitive-binding ELISA were indicative of similar displacement curves for free LL2 and emulsion-LL2 conjugates. Direct cellular ELISA revealed similar binding of emulsion-LL2 complexes to three types of Burkitt's lymphoma cell lines, Raji, Ramos and Daudi. The results from this study indicate that emulsion-LL2 complexes might be a useful drug-carrier system for more specific delivery of anticancer drugs to B-cell malignancy.
PMID: 10579680 [PubMed - indexed for MEDLINE]

Finally, Applicants note that Lundberg et al. disclose coupling of an LL2 antibody to lipid emulsions. Applicants submit that the LL2 antibody, which binds to CD22, is distinct from the anti-CD74 antibodies claimed herein, and that the skilled artisan would not be motivated to substitute LL1 for LL2 to make the claimed combination.

Attached herewith is an in press manuscript by Stein et al., "CD74: A New Candidate Target for the Immunotherapy of Hematological Malignancies," which discusses the significant differences between anti-CD74 and other anti-B cell antibodies. Specifically, the paragraph bridging pages 6-7 states that:

> B-cell lymphomas were found to take up and catabolize nearly 10^7 LL1 molecules per day, a much faster rate than that of other antibodies that are considered to be rapidly internalized, such as CD19, CD22, and anti-transferrin-receptor antibodies (31). Moreover, the internalization of LL1 differs from that of the anti-growth factor receptors, another class of mAbs showing rapid internalization (including anti-epidermal growth factor (EGF) receptor), because it is not dependent on antibody crosslinking. An additional notable difference is that with anti-EGF-receptor, anti-transferrin receptor, and anti-CD22 antibodies, receptor expression is down-regulated after antibody binding. Thus, large amounts of anti-CD74 antibody, but not of the other internalizing mAbs, are transported into the target cells.

The first full paragraph on page 8 recites:

> In comparisons of hLL1 to the chimeric anti-CD20 MAb, rituximab, we observed that the two antibodies act through distinct mechanisms and exhibit different expression and sensitivity profiles on B-cell malignancies (23). As with rituximab, in most human lymphoma or MM cell lines, hLL1 alone does not show a direct cytotoxic effect in vitro. However, in the presence of an appropriate crosslinking agent, hLL1 causes inhibition of cell proliferation and induces apoptosis. Unlike rituximab, hLL1 induces little or no antibody-dependent cellular cytotoxicity (ADCC) or complement-mediated cytotoxicity.

At page 11, second paragraph the manuscript concludes that:

> The unique property of LL1 is its high level of intracellular uptake. Because of this conjugates of Auger electrons and LL1 can be used to deliver a greater quantity of Auger electrons than other conjugated mAbs. *In vitro*, B-lymphoma cells are efficiently and specifically killed by LL1 conjugated to various radiolabels, including ^{111}In, ^{125}I, ^{99m}Tc, ^{67}Ga, ^{131}I, and ^{90}Y (36, 37). Total killing was obtained (greater than 6 logs) under conditions in which a non-reactive antibody labeled in the same way produced no significant toxicity. Because of the rapid internalization and catabolism of LL1, radioiodine conjugates were only active if "residualizing" forms were used (i.e., forms which are trapped inside the cell after catabolism of the antibody). Conventional oxidative iodination produces a non-residualizing label in which the radio-iodotyrosine produced after internalization and catabolism of labeled mAb diffuses from the target cell precluding accumulation of isotope (37). Conjugates of LL1 with the β-particle emitters yielded more nonspecific toxicity in *in vitro* studies than Auger-emitters, and correspondingly, much lower doses of β-particle emitting isotopes could be administered in an *in vivo* model of tumor growth.

Thus, Applicants submit that anti-CD74 antibodies, such as LL1, show significant differences in their internalization properties, toxicities and therapeutic effects when compared with other anti-B cell antibodies such as anti-CD22 or anti-CD20. For these reasons, the skilled artisan would have had no reasonable expectation of success in substituting an anti-CD74 antibody for the anti-CD22 antibody discussed in Lundberg et al.

The Action asserts that, "Hansen et al. teaches that the LL1 antibody is rapidly internalized on cells expressing the MHC I invariant chain as measured by a 111-In chelate of DTPA. Hansen et al suggest that the LL1 antibody is useful for the delivery of toxins, drugs or radioisotopes that can kill tumor cells expressing surface Ia, such as B cell lymphomas." (citations omitted)

The Action concludes that it would have been obvious to substitute the anti-CD74 LL1 antibody of Hansen et al. for the LL2 antibody of Lundberg et al. Applicants first note that with

the proper priority date, Lundberg is not prior art to the instant claims. Therefore, there could have been no combination between the disclosures of Hansen and Lundberg. Further, as discussed above, the properties of anti-CD74 antibodies, such as LL1, differ significantly from those of other B-cell antibodies, such as LL2. Therefore, the skilled artisan could have had no reasonable expectation of success in making the substitution.

To summarize, Juweid did not disclose the conjugation of anti-CD74 antibodies to lipid emulsions, micelles, polymeric carriers or nanoparticles, as asserted by the Action, and Lundberg is not prior art to the proper priority date of the instant claims. Thus, none of the prior art, alone or together, teaches or suggests the claimed element of a composition comprising one or more anti-CD74 antibodies or fragments conjugated to one or more lipids, polymeric carriers, micelles or nanoparticles, in combination with one or more effectors. In the absence of this claim element, a *prima facie* case of obviousness has not been established under MPEP §2142 and rejection under 35 U.S.C. §103 is improper. Reconsideration and withdrawal of the rejection are requested.

Claims 1-10, 13-21, 27, 35-38 and 125 are rejected under 35 U.S.C. 103(a) as unpatentable over Pawlak-Byczkowska, Juweid, Lundberg and Hansen and further in view of Schlom (In: Molecular Foundations of Oncology, Samuel Broder, Ed.,1991, pages 95-34). Schlom is said to teach that in human trials with non-immunosuppressed patients treated with multiple doses of murine antibodies, only the first and possibly second doses efficiently reach the tumor site because of HAMA. Schlom is further said to teach humanization of the murine antibody to reduce HAMA.

The deficiencies of Pawlak-Byczkowska, Juweid, Lundberg and Hansen are discussed above. Schlom does nothing to cure those deficiencies, as it merely relates to humanization of antibodies and contains no teaching, disclosure or suggestion of the claimed element of a composition comprising one or more anti-CD74 antibodies or fragments conjugated to one or more lipids, polymeric carriers, micelles or nanoparticles, in combination with one or more effectors. Because this claim element is missing from all of the cited prior art, a *prima facie* case of obviousness has not been established. Reconsideration and withdrawal of the rejection are requested.

Claims 1-10, 13-21, 27, 35-38, 40 and 125 are rejected under 35 U.S.C. 103(a) as unpatentable over Pawlak-Byczkowska, Juweid, Lundberg, Hansen and Schlom in view of

Greenwood et al. (In: Protein engineering of antibody molecules, for therapeutic and prophylactic applications in man, Clark, ed., 1993, pages 89 and 97 "Greenwood"). The Action asserts that Greenwood teaches use of antibody isotypes that are non-depleting and target IgG3, IgG2 or IgG3.

The deficiencies of Pawlak-Byczkowska, Juweid, Lundberg, Hansen and Schlom are discussed above. Greenwood does nothing to cure those deficiencies, as it contains no teaching, disclosure or suggestion of the claimed element of a composition comprising one or more anti-CD74 antibodies or fragments conjugated to one or more lipids, polymeric carriers, micelles or nanoparticles, in combination with one or more effectors. Because this claim element is missing from all of the cited prior art, a *prima facie* case of obviousness has not been established. Reconsideration and withdrawal of the rejection are requested.

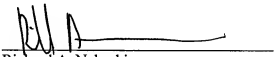
Claims 1, 2, 6, 8, 11, 20, 21, 24-26, 28, 29, 35 and 125 are rejected under 35 U.S.C. 103(a) as unpatentable over Juweid in view of Goto et al. (Blood 1994, 84:1922-30, "Goto"). The Action asserts that Juweid teaches, "a 99Tcm-LL1 antibody conjugate in a sulfur colloid as a carrier (abstract) which meets the limitations of" claims 1, 2, 6 and 8. Goto is said to "teach an additional B cell restricted antiben, HMI-24 which is a target for late-stage B cell maturation and multiple myeloma."

The deficiencies of Juweid are discussed above. As discussed above, Juweid does not teach a 99Tcm-LL1 antibody conjugate in a sulfur colloid as a carrier. Goto does nothing to cure the deficiencies of Juweid, as it contains no teaching, disclosure or suggestion of the claimed element of a composition comprising one or more anti-CD74 antibodies or fragments conjugated to one or more lipids, polymeric carriers, micelles or nanoparticles, in combination with one or more effectors. Because this claim element is missing from all of the cited prior art, a *prima facie* case of obviousness has not been established. Reconsideration and withdrawal of the rejection are requested.

Conclusion

For the reasons stated above, Applicants submit that the amended claims are in condition for allowance and request withdrawal of the rejections.

Respectfully submitted,



Dated: June 18, 2007

Richard A. Nakashima
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1: Vopr Onkol. 1994;40(7-12):314-8.

Related Articles, Links

[Immunocyte subpopulations in histologic sections of breast tumors]

[Article in Russian]

Tupitsyn NN, Artamonova EV, Kadagidze ZG, Blokhina NG, Baryshnikov AI, Moldenhauer H, Boumcelle L.

A study of tumor infiltrating lymphocytes was carried out on cryostat sections of breast tumors in 27 patients using 24 monoclonal antibodies. Two types of infiltration--stromal and intraparenchymal--were identified. Certain correlations characteristic of subpopulation levels of immunocompetent cells identifiable by monoclonal antibody clusters CD8, CD22, CD38 and CD7; CD74 and CD4; CD11b and CD22, CD37 were established. The study also established a correlation between levels of immunocompetent cells infiltration (CD4+, CD7+, CD8+ and CD11b+) and tumor size and regional lymph node involvement.

Publication Types:
- English Abstract

PMID: 7610627 [PubMed - indexed for MEDLINE]

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1: Mol Immunol. 1994 Dec;31(17):1365-8.

Related Articles, Links

Retinoblastoma protein regulation of surface CD74 (invariant chain) expression in breast carcinoma cells.

Lu Y, Ussery GD, Jacim M, Tschickardt M, Boss JM, Blanck G.

Department of Biochemistry and Molecular Biology, University of South Florida College of Medicine, Tampa 33612.

The HLA class II genes encode heterodimeric cell surface proteins which bind peptide antigen recognized by T-cell receptors on CD4+ T-cells. The class II proteins are inducible by IFN-gamma, and this induction requires, or is strongly enhanced, by retinoblastoma protein (RB) in a series of breast carcinoma cell lines. Loading of peptide onto the class II protein appears to be regulated by CD74, which associates with class II during their transition to the endosomal compartment, where class II binds peptide. Class II proteins and CD74 are largely regulated in concert, provoking the question, is CD74 induction by IFN-gamma affected by RB? Results described here indicate that IFN-gamma induction of CD74 surface expression in a series of breast carcinoma lines is enhanced by RB, while RB has no effect on CD74 mRNA induction. Also, neither the class II nor the CD74 promoter regions are activated by RB in cotransfection experiments where RB activates the SV40 promoter.

Publication Types:
- Research Support, Non-U.S. Gov't

PMID: 7997248 [PubMed - indexed for MEDLINE]

1: Am J Surg Pathol. 1996 Jan;20(1):64-71.

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Related Articles, Links

Lymphoid monoclonal antibodies reactive with lung tumors. Diagnostic applications.

Ioachim HL, Pambuccian SE, Hekimgil M, Giancotti FR, Dorsett BH.

Department of Pathology, Lenox Hill Hospital, New York, NY 10021, USA.

In the course of investigating 30 monoclonal antibodies (MAbs) for their potential reactivity with 25 lung tumors of different histologic types, we found that three MAbs commonly used for their specificities for lymphoid markers were highly reactive with non-small-cell carcinomas (NSCLC) and totally nonreactive with small-cell carcinomas (SCLC). Immunostaining was performed by the standard streptavidin-biotin-peroxidase method after microwave antigen retrieval on formalin-fixed, paraffin-embedded tissue sections. LN2 (CD74), LN3 (HLA-DR), and BLA-36, which are commonly used for the identification of B-lymphocytes, strongly immunostained 19 of 25 squamous and adenocarcinomas and none of 34 small-cell carcinomas and carcinoids. Moreover, in combined tumors, these MAbs selectively stained the adenocarcinoma cells but not the adjacent small-cell carcinoma cells. A cocktail mixture of LN2, LN3, and BLA-36 assayed on 24 additional lung tumors produced similar results with even stronger and sharper stainings. Other lymphoid MAbs showed some selective staining but to a lesser degree. Among nonlymphoid MAbs, the results were as expected, with MAbs for cytokeratin (B72.3) and epithelial membrane antigen staining NSCLC but also some SCLC. The MAbs for chromogranin and neuron-specific enolase were not entirely specific, whereas some nerve-cell adhesion molecule MAbs showed good specificity for SCLC. In a field with few specific MAbs, the newly discovered ability of these lymphoid MAbs to discriminate between SCLC and NSCLC may prove useful in the immunohistochemical diagnosis of lung tumors.

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1: Nippon Hinyokika Gakkai Zasshi. 1993 Jun;84(6):1036-40.

Related Articles, Links

[Expression of HLA class II antigen-associated invariant chain on renal cell cancer]

[Article in Japanese]

Saito T, Tomita Y, Kimura M, Nishiyama T, Sato S.

Department of Urology, Niigata University, School of Medicine.

Major histocompatibility complex (MHC) antigens play important roles in immune responses. MHC class II molecules serve as restriction elements for cells presenting antigens to CD4-positive helper T-cells. They also function as histocompatibility antigens responsible for graft rejections by stimulating allogenic reaction. Furthermore, it was reported that the expression of class II antigen on tumor cells increases immunogenicity in mice by a mechanism involving class II molecule positive tumor cells which present tumor associated antigens to host immune cells or induce allogenic reactions against tumor cells. Class II molecules associate with the HLA class II antigen-associated invariant chain (Ii) as soon as class II molecules are produced in the rough endoplasmic reticulum. Ii is considered to block the binding of endogenous peptides to class II molecules, and to be a signal for transporting class II molecules to endosomes. Both functions of Ii are essential for the function of class II molecules. To investigate the association between renal cell cancer (RCC) and the host's immune system, we immunohistochemically examined 60 RCCs for the expression of Ii, class II and class I antigens on RCC and the degree of lymphocytic infiltration. We detected Ii to varying degrees on 53 out of 60 RCC tissues but none on normal renal tubular cells. Compared with class II antigens, they were detected in the order DR > or = Ii > or = DP not equal to DQ. A significant correlation was found between the expression of Ii and the degree of lymphocytic infiltration.(ABSTRACT TRUNCATED AT 250 WORDS)

Publication Types:
- English Abstract

CD74: A New Candidate Target for the Immunotherapy of Hematological Malignancies

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Key words: CD74, lymphoma, monoclonal antibody, multiple myeloma, therapy

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ABSTRACT

CD74 is an integral membrane protein that functions as an MHC class II chaperone. Moreover, it has recently been shown to have a role as an accessory signaling molecule and has been implicated in malignant B-cell proliferation and survival. These biological functions combined with expression of CD74 on malignant B-cells and limited expression on normal tissues combine to implicate CD74 as a potential therapeutic target. The anti-CD74 monoclonal antibody, LL1, has been humanized (hLL1 or IMMU-115), and can provide the basis for novel therapeutic approaches to B-cell malignancies, particularly since this antibody shows rapid internalization into CD74+ malignant cells. This paper reviews the preclinical evaluations of LL1, its humanized form, and isotope-, drug-, and toxin-conjugates. These studies show that unconjugated hLL1 and conjugates of hLL1 constructs with radioisotopes, doxorubicin, and frog RNase have high anti-tumor activity in non-Hodgkin lymphoma and multiple myeloma *in vitro* and in tumor xenograft models. Single-dose studies of hLL1 in monkeys showed no adverse effects, but did decrease circulating B- and T-lymphocytes, and NK cells. When evaluated in combination with rituximab, either equivalent or improved efficacy, as compared to either antibody alone, was observed. **Conclusion:** CD74 is a new candidate target for the immunotherapy of neoplasms expressing this antigen, which can be exploited using either a naked antibody or conjugated to isotopes, drugs, or toxins.

INTRODUCTION

CD74 (invariant chain, Ii) is a type II transmembrane glycoprotein that associates with the major histocompatibilty (MHC) class II α and β chains and directs the transport of the $\alpha\beta$Ii (invariant chain) complexes to endosomes and lysosomes (1-4). In addition, CD74 is involved in signaling pathways functioning as a survival receptor (5). These biological functions combined with expression of CD74 on malignant B-cells and limited expression on normal tissues implicate CD74 as a potential therapeutic target. The anti-CD74 monoclonal antibody, LL1, has been humanized (hLL1 or IMMU-115), and can provide the basis for novel therapeutic approaches to B-cell malignancies. This paper reviews the preclinical evaluations of LL1, its humanized form, and isotope-, drug-, and toxin-conjugates. The evidence suggests that hLL1 is a promising candidate antibody for the therapy of CD74-expressing malignancies, such as non-Hodgkin lymphoma (NHL) and multiple myeloma (MM). Due to its rapid internalization property, hLL1 also shows considerable promise as a drug, isotope, or toxin immunoconjugate.

CD74 Molecule: structure, function, expression

CD74 has 30 NH_2-terminal, intracytoplasmic amino acid residues, a 26 amino acid hydrophobic transmembrane region, and a 160 amino acid extracytoplasmic domain containing two N-linked carbohydrate chains (1, 6). Once synthesized, CD74, DRα, and DRβ begin to associate within the endoplasmic reticulum. This is believed to occur by sequential addition of DRα/β heterodimers to a trimeric core of CD74 molecules until a nine-subunit complex with equimolar amounts of these three chains is formed (7, 8). DR-CD74 complexes then

progressively traffic to the late endosomal compartment where CD74 is cleaved into peptide fragments by proteases. These CD74 cleavage fragments have reduced affinity for DR, and allow for the displacement of CD74 by peptides present in the endosome. The DR-peptide complexes then traffic to the cell surface for antigen presentation. During this process, a sizable DR-CD74 pool transiently resides on the cell surface.

In addition to its role as a chaperone molecule for MHC Class II, CD74 has been shown to function as a signaling molecule in several pathways. CD74 was shown to be directly involved in the maturation of B-cells through a pathway involving NF-κB (9, 10). Using activating anti-CD74 mAbs, Startlett et al. (5), demonstrated recently that syk, PI3 kinase, and AKT are activated following ligation of CD74 on murine B-cells, and that activation of NF-κB occurs with downstream transcription of anti-apoptotic genes such as Bcl-X_L. CD74 has also been shown to be the high affinity receptor for the proinflammatory cytokine, macrophage migration-inhibitory factor (MIF) (11). MIF is ubiquitously expressed and contributes to innate immune system activation in macrophages and monocytes through activation of the ERK signaling pathway (11, 12). Numerous reports have shown that MIF inhibits p53 function (13, 14), activates components of the mitogen-activated protein kinase and Jun-activation domain-binding protein-1 (Jab-1) pathways (12, 15, 16), antagonizes the action of glucocorticoids (17), and upregulates Toll-like receptor 4 (TLR-4) (18). MIF serves as both an accelerant of malignant lymphoid disease and contributor to the neovascularization necessary for tumor development, and therefore interfering with MIF-associated pathways may have anti-lymphoma effects (14). Recently, CD44 has been reported to be an integral member of the CD74 receptor complex

leading to MIF signal transduction (19), substantiating earlier reports of a functional and cell surface association between CD74 and CD44 (20).

In normal tissues, CD74 is expressed on HLA class II-positive cells, including B cells, monocytes, macrophages, Langerhans cells, dendritic cells, subsets of activated T-cells, and thymic epithelium. Under inflammatory conditions, CD74 expression may be observed on endothelial and certain epithelial cells (21). CD74 is also expressed on a variety of malignant cells. Its expression has been observed in ~90% of B-cell cancers evaluated, as well as the majority of cell lines derived from these cancers (22, 23). Table 1 summarizes immunohistochemical staining of patient biopsy specimens with the anti-CD74 mAb, LL1. Staining of a trephine bone biopsy from a MM patient with the anti-CD74 mAb LL1 is shown in Figure 1. CD74 staining is seen in greater than 95% of MM plasma cells. Malignant plasma cells from adjacent histologic sections were identified with an anti-CD138 mAb. This specimen was among a group of 22 such biopsy specimens tested for CD74 expression. Nineteen of these 22 specimens exhibited positive staining, with 16 showing greater than 95% of the MM cells strongly reactive, as exemplified in Figure 1A. This group of cases was also assessed for HLA-DR expression. As seen in Figure 1B less staining was seen with the anti-HLA-DR mAb (TAL.1B5) in the MM cases. This is in contrast with most normal and malignant cell types, which co-express DR and CD74. This finding may have implications for the antigenicity of MM specimens with respect to their recognition by the host immune system.

CD74 expression has also been found in non-hematologic malignancies, including gastric (24), renal (25), non-small-cell lung (26), and thymic epithelium neoplasms (27), certain types of

sarcoma (fibrous histiocytoma) (28), and atypical fibroxanthoma, an unusual malignant fibrohistiocytic tumor of sun-damaged skin (29). CD74 expression in many of these cancers has been suggested to be a prognostic factor, with higher relative rates of CD74 behaving as a marker of tumor progression. This correlation may be related to suppressive effects on host immune responses.

Anti-CD74 monoclonal antibody, LL1

The murine LL1 mAb (IgG1κ) was generated by hybridoma technology after immunization of BALB/c mice with Raji human Burkitt lymphoma cells (30).The CD74 specificity of LL1 was first suggested by immunoflourescence and immunohistology studies that showed discrimination between lymphoid and non-lymphoid tissues, reactivity with malignant B-cell tissues sections and cloned B-cell lymphomas, and molecular size determinations by Western immunoblots of cell extracts. Confirmation that LL1 binds to a cell-surface-expressed epitope of CD74 was then provided by competitive binding experiments and immunoprecipitation studies in conjunction with other anti-CD74 antibodies. Binding was inhibited by mAb LN2, which reacts with a cell surface epitope, but not BU45 or POP.I4.3, which do not. LL1 is unique in that although it reacts with the LN2 epitope its cell surface binding is markedly stronger than that of LN2 (31).

One of the most interesting properties of LL1 was uncovered by following the fate of the antibody after binding to the cell surface. B-cell lymphomas were found to take up and catabolize nearly 10^7 LL1 molecules per day, a much faster rate than that of other antibodies that

are considered to be rapidly internalized, such as CD19, CD22, and anti-transferrin-receptor antibodies (31). Moreover, the internalization of LL1 differs from that of the anti-growth factor receptors, another class of mAbs showing rapid internalization (including anti-epidermal growth factor (EGF) receptor), because it is not dependent on antibody crosslinking. An additional notable difference is that with anti-EGF-receptor, anti-transferrin receptor, and anti-CD22 antibodies, receptor expression is down-regulated after antibody binding. Thus, large amounts of anti-CD74 antibody, but not of the other internalizing mAbs, are transported into the target cells. Studies with other CD74-expressing cell types, including interferon-stimulated melanoma and colon carcinoma cell lines, supported the observations made in the lymphoma cells. In all the tested cell lines, CD74 internalized rapidly, while being replaced by newly synthesized molecules, resulting in a high cumulative expression of CD74 on the surface, even though the steady-state level was relatively low (32). Because the rapid internalization of LL1 provides rapid delivery of large numbers of molecules to lysosomes, it made LL1 an especially interesting candidate for the delivery of toxins, drugs, or radioisotopes that can kill CD74-expressing tumor cells.

Humanized LL1

A humanized form of LL1, hLL1, was generated by complementarity-determining region (CDR) grafting, and exhibited comparable antigen-binding and internalization properties as the parental murine antibody (23, 33). The humanized anti-CD74 MAb, hLL1, causes specific *in vitro* and growth inhibition and induction of apoptosis in B-cell lines in the presence of a second crosslinking antibody. In addition, significant survival extensions were observed in NHL- and

MM-bearing SCID mice treated with naked hLL1 without the need for an exogenous crosslinking agent.

In comparisons of hLL1 to the chimeric anti-CD20 MAb, rituximab, we observed that the two antibodies act through distinct mechanisms and exhibit different expression and sensitivity profiles on B-cell malignancies (23). As with rituximab, in most human lymphoma or MM cell lines, hLL1 alone does not show a direct cytotoxic effect in vitro. However, in the presence of an appropriate crosslinking agent, hLL1 causes inhibition of cell proliferation and induces apoptosis. Unlike rituximab, hLL1 induces little or no antibody-dependent cellular cytotoxicity (ADCC) or complement-mediated cytotoxicity. Antiproliferative effects of hLL1 and rituximab on a panel of NHL and MM cell lines are shown in Figure 2. Incubation with hLL1 caused specific inhibition of proliferation in 5 of 6 NHL cell lines in the presence of a crosslinking second antibody (Figure 2A). The sensitivity profile of the cell lines differed for rituximab (Figure 2B). For example, in the absence of crosslinking, no anti-proliferative activity was seen with hLL1 in any cell line, whereas rituximab yielded approximately 23 – 88% inhibition of Daudi, Ramos cells, and SU-DHL-6 cells. However, significant augmentation of the anti-proliferative effects of rituximab was also observed with crosslinking. Interestingly, the variation in level of inhibition was not correlated strictly to antigen density. For example, in WSU-FSCCL, CD74 antigen density is half that of CD20; however, [^{3}H]-thymidine incorporation was inhibited 80% by hLL1+GAH (goat anti-human) IgG crosslinking, whereas these cells were unaffected by rituximab + GAH IgG. In MM cell lines, the antiproliferative activity of hLL1 and rituximab correlated with the presence of antigen in this cell line panel (Figure 2C and 2D).

ARH-77 and MC/CAR, CD20+ and CD74+ cell lines, were sensitive to both hLL1 and rituximab; CAG and KMS12-PE, CD20-, CD74+, were only inhibited by hLL1; and the two CD20-, CD74- cell lines, ARK and ARD, were insensitive to both MAbs.

Importantly, anti-proliferative activity can be augmented when anti-CD74 and anti-CD20 MAbs are combined (23). Studies were conducted using hLL1 combined with rituximab, as well as IMMU-106, a humanized anti-CD20 mAb (34). As shown in Table 2, the combination of hLL1 and anti-CD20 mAb was equal to or greater than that of the single crosslinked mAb treatments in all cell lines tested. Similar results were obtained with rituximab or IMMU-106. This observation is important, because it is expected that in humans, IMMU-106 should be at least as effective as rituximab, and because of its human framework, it may exhibit different pharmacokinetics, toxicity, and therapy profiles than the chimeric rituximab (34).

In vivo, therapeutic efficacy of hLL1 was demonstrated in SCID mice using NHL and MM cell lines. In the human Burkitt lymphoma xenografts, Daudi and Raji, median survival was extended significantly; a 45% increase was obtained in Raji and a 19% increase in Daudi-bearing SCID mice given lower doses. In both studies, treatments were initiated one day after injection of tumor cells (23). Therapeutic efficacy was markedly greater in the MC/CAR MM cell line. Figure 3 shows the results of a study comparing the efficacy of hLL1 in MC/CAR-bearing SCID mice treated by 4 dose schedules. Median survival of untreated mice was 28 days. The various treatment schedules initiated 5 days after tumor cell injection extended median survival to 49-112 days. Initiation of hLL1 dosing one day after tumor cells were inoculated yielded long-term

survival (>150 days) in 8 of 10 mice. No treatment-related toxicities were observed, as measured by body weight loss (23). Similar efficacy has recently also been observed in WSU-FSCCL follicular lymphoma and CAG MM xenografts demonstrating the broad usefulness of naked hLL1 against B-cell malignancies (data not shown).

The hLL1 safety profile was evaluated by performing toxicology studies in Cynomolgus monkeys, because hLL1 cross-reacts with Cynomolgus monkey CD74. Up to 125 mg/kg were administered in a single dose escalation scheme and up to 50 mg/kg were administered in a multi-dose escalation scheme (Phase I: a single dose followed by a 4-week observation period; Phase II: twice-weekly doses for 4 weeks). No test article related mortality was observed at any dose. In addition, there were no overt evidence of systemic toxicity to any major organ, and clinical observations and clinical pathology were normal. Expected decreases were noted in PBMCs, including B- and T-lymphocytes, NK cells, and plasma cells, following the multi-dose scheme. None of the changes persisted through the 1-week or 4-week recovery periods. (Immunomedics, Inc., data on file.)

Radiolabeled Antibody

Although radioimmunotherapy has now been under investigation for over two decades, there has not been a consensus as to the choice of optimal radioisotope. Indeed, the choice of isotope depends on the specific application (tumor burden and tumor type), and specific tradeoffs between efficacy and toxicity must be made. The high energy β–emitters, such as ^{131}I and ^{90}Y, have been widely used to kill large tumor masses. However, the radiation dose delivered is

primarily due to crossfire from neighboring cells and can cause significant toxicity, especially in bone marrow. In addition, such radiation is unable to efficiently kill single cells. This is significant clinically because it implies that mAbs carrying ^{131}I and ^{90}Y cannot efficiently kill micrometastases or single tumor cells. α-Particles can kill single cells effectively due to their relatively short path length and high energy. However, the available α-particle-emitters have short half-lives (≤7 h), and are therefore unsuitable for treatment of solid tumors because of the substantial time required for tumor penetration, but may be of value for micrometastatic disease (35). Auger electron-emitting radionuclides are a third category of toxic isotopes. The low energy of the emissions requires the isotope to be in close proximity to the nucleus, or present at high concentration. When these conditions are met, Auger electron-emitting radionuclides are suitable for killing single cells and micrometastases.

LL1 has been evaluated with all three of these types of radioisotopes. The unique property of LL1 is its high level of intracellular uptake. Because of this conjugates of Auger electrons and LL1 can be used to deliver a greater quantity of Auger electrons than other conjugated mAbs. *In vitro*, B-lymphoma cells are efficiently and specifically killed by LL1 conjugated to various radiolabels, including ^{111}In, ^{125}I, ^{99m}Tc, ^{67}Ga, ^{131}I, and ^{90}Y (36, 37). Total killing was obtained (greater than 6 logs) under conditions in which a non-reactive antibody labeled in the same way produced no significant toxicity. Because of the rapid internalization and catabolism of LL1, radioiodine conjugates were only active if "residualizing" forms were used (i.e., forms which are trapped inside the cell after catabolism of the antibody). Conventional oxidative iodination produces a non-residualizing label in which the radio-iodotyrosine produced after internalization and catabolism of labeled mAb diffuses from the target cell precluding

accumulation of isotope (37). Conjugates of LL1 with the β-particle emitters yielded more nonspecific toxicity in *in vitro* studies than Auger-emitters, and correspondingly, much lower doses of β-particle emitting isotopes could be administered in an *in vivo* model of tumor growth.

A mouse xenograft model, consisting Raji human B-cell lymphoma cells injected i.v. into SCID mice, was used to determine whether specific therapeutic effects could be demonstrated *in vivo* with LL1 conjugates of ^{111}In, ^{67}Ga, and ^{90}Y (38). Radiolabeled mAbs were injected at various times after tumor inoculation. Tumor growth was monitored by hind-leg paralysis. With a 3-5 day interval before antibody injection, LL1 conjugated to the Auger emitters (^{111}In and ^{67}Ga) at a dose of 240-350 μCi/mouse yielded a strong therapeutic effect, with greatly delayed tumor growth. Moreover, many of the treated mice were tumor-free for greater than six months, whereas control mice became paralyzed in 16-24 days. With ^{90}Y-labeled LL1, the maximal tolerated dose was markedly lower, 25 μCi/mouse, and at this dose there was only a weak therapeutic effect. Effective and specific therapy of disseminated B-cell lymphoma in SCID mice with ^{111}In-labeled LL1 is shown in Figure 4A. LL1 increased median survival almost 7-fold, from 18 to 124 days, and produced a complete cure (6 months without evidence of tumor growth) in 40% of the mice. A dose-response experiment with ^{111}In-LL1 is shown in Figure 4B, where treatment was given 5 days after tumor cells in this study. Median survivals of 19, 28, 40, and 115.5 days were observed, respectively, in the untreated, 31 μCi, 92 μCi, and 275 μCi ^{111}In-LL1 treatment groups. Experiments in which the interval between tumor injection and mAb administration was varied are shown in Figure 4C. During the six-month observation period, only 1 of 10 mice died (at day 155) when 350 μCi ^{111}In-LL1 was given three days after cells. Median survivals of 124 days and 29 days were obtained with 5- and 9-day intervals,

respectively. No significant toxicity was observed. Unlabeled LL1 administered at the same concentration and dose schedule as the radiolabeled preparations did not have a significant effect on survival. Thus, under these conditions, the therapeutic effects observed were attributable to the radioactivity and not the mAb itself. Moreover, a similarly strong therapeutic effect was observed using the MTD (240 μCi/mouse) of ^{67}Ga-LL1. In contrast, ^{90}Y-labeled LL1 used at the MTD (25 μCi/mouse) had only a small therapeutic effect; the median survival time was increased only from 18 to 29 days. ^{111}In-LL1 was also observed to be an effective therapeutic agent in a study of small subcutaneous B-lymphoma xenografts, extending the utility of this agent to a somewhat larger tumor size (39).

The α-emitter ^{213}Bi,with a half-life of 46 min, was conjugated to LL1 with the chelator CHX-A"-DTPA. The conjugate was an effective and specific toxic agent for B-cell lymphoma, both *in vitro* and *in vivo* in the SCID mouse Raji cell xenograft micrometastatic model (35). Although the short half-life limits the usefulness of ^{213}Bi-mAb conjugates for solid tumors, they may be useful for treatment of accessible tumors, such as leukemia cells or micrometastases, where antibody binding is rapid. In addition, α-particle emitters may be useful with antibody fragments, due to the faster tumor penetration of the lower molecular weight fragments. Progress in the use of α-particle emitters has been made and there are on-going studies which may bring additional enthusiasm to their use in radioimmunotherapy (40, 41).

These observations demonstrate that LL1 is an effective agent for delivery of radionuclides, including both Auger (^{111}In, ^{67}Ga, ^{125}I) and α-particle (^{213}Bi) emitters. ^{111}In- and ^{67}Ga-labeled LL1 were markedly superior to ^{90}Y-LL1, suggesting that these isotopes are more

effective than conjugates with β-particle emitters for the treatment of micrometastases. This was corroborated in a recent study of ^{177}Lu-LL1 (42).The potency of LL1 labeled with Auger electron-emitting radioisotopes is due to the remarkably high level of accumulation within cells.

Drug and Toxin Antibody Conjugates

Conjugates of mAbs with drugs or toxins have been investigated for many years as a potential approach to delivering these agents more specifically to cancers (43). Although it was contended previously that conventional chemotherapy drugs, such as doxorubicin (dox), had inadequate potency to elicit a clinical effect (44-47), the rapid internalization of LL1 led us to reevaluate this concept using this antibody. The chemical properties of dox and similar anthracyclines are favorable for protein linkage. Specifically, anthracyclines are water soluble, have appropriate hydrophobicity, and have several reactive groups usable as linkage sites. Finally, dox is a known chemotherapeutic, acting by several mechanisms, including inhibition of topoisomerase II, intercalation into DNA, and effects on cell membranes (48, 49).

hLL1-dox (IMMU-110), is a drug immunoconjugate comprised of dox conjugated to hLL1, at 6-8 drug molecules per IgG molecule. The dox is linked to partially reduced antibody inter-chain disulfide groups using 4-[N-maleimidomethyl] cyclohexane-1 carboxylhydrazide. Evaluation of hLL1-dox and murine LL1-dox in cell lines and tumor-bearing SCID mice demonstrated very high anti-tumor activity in both NHL and MM models. *In vitro* toxicity of hLL1-dox is shown in Figure 5. Activity of hLL1-dox was evaluated in comparison to a negative control dox conjugate (hMN-14-dox) (50). Toxicity of hLL1-dox was similar to that of free dox in CD74+ cell lines, whereas the control conjugate was markedly less toxic (51). In CD74- cell

lines, toxicity of hLL1-dox was similar to the negative control conjugate. *In vivo* efficacy is shown in Figure 6. Results of single dose treatments given 5 days after administration of tumor cells are shown in Figure 6A for a NHL model (Raji) and Figure 6B for a MM model (Mc/CAR). At 350 μg/animal, hLL1-dox led to long term survival of 100% of Raji-bearing and 60% of MC/CAR-bearing mice, whereas no significant effects were seen with unconjugated dox given at an equivalent dose, or control conjugates (52, 53). The naked hLL1 had significant anti-tumor effects in the MM model, but less so than the drug-immunoconjugate. Similar survival extensions were observed when treatment with hLL1-dox was given up to 10 days after transplantation of MM or NHL cells, and significant, but smaller, survival extension were even observed 14 days post-tumor cell inoculation. The efficacy of hLL1-dox was confirmed in other studies in SCID mice bearing the Burkitt lymphoma Daudi (53) and the follicular lymphoma WSU-FSCCL (data not shown).

In SCID mice, hLL1-dox failed to show any toxicity, including myelotoxicity and cardiotoxicity, up to the maximum single dose tested of 2.5 mg/mouse (protein dose = 125 mg/kg; dox equivalent dose = 3.6 mg/kg), although the MTD of free dox was reached at 3.6 mg/kg. Therefore, conjugation of dox to hLL1 mitigated the toxicity of free dox in SCID mice. Lack of cross-reactivity with rodent CD74 makes safety studies in mice less relevant. Therefore, preliminary tolerability studies of hLL1-dox in nonhuman primates (cynomolgus monkeys) were performed, because hLL1 reacts with monkey CD74. Although these acute toxicity studies were done in a relatively small number of animals, they suggest that hLL1-dox is well tolerated up to 30 mg/kg, at which level the first signs of bone marrow toxicity appeared. No acute

cardiotoxicity or signs of adverse effects to other major organs were observed at doses up to 90 mg/kg (53).

An excellent therapeutic index was also achieved in preclinical models of NHL with a recombinant fusion protein of humanized anti-CD74 and the toxin Ranpirnase (54). Ranpirnase (Rap) is an amphibian ribonuclease (RNase) belonging to the RNase A superfamily (55). Rap has a low affinity for the RNase inhibitor present at high levels in mammalian cells and therefore evades inactivation. Rap enters cells via receptor-mediated endocytosis, and once internalized selectively degrades tRNA, resulting in inhibition of protein synthesis and induction of apoptosis (56-58). A novel recombinant ribonuclease-anti-CD74 humanized IgG4 antibody immunotoxin, 2L-Rap-hLL1-γ4P, composed of 2 Rap molecules, each fused to the N terminus of the light chain of hLL1 was designed and expressed. To reduce unwanted side effects caused by γ1 effector cell functions, the constant region of hLL1 was changed from γ1 to γ4. In addition, a point mutation, Ser241Pro, was introduced into the hinge region of the γ4 sequence to avoid formation of half-molecules when the antibody is expressed and produced in mammalian cell cultures (59). *In vitro*, 2L-Rap-hLL1-γ4P retained RNase activity, specific binding to CD74, and was significantly more potent against NHL and MM CD74+ cell lines than naked hLL1. *In vivo*, the pharmacokinetics profile of 2L-Rap-hLL1-γ4P was similar to that of hLL1. Most importantly, marked therapeutic efficacy was shown in animal models of B-cell lymphomas (Daudi and Raji), where treatment with a single 5 to 50 μg dose of 2L-Rap-hLL1-γ4P, given in early or advanced disease, resulted in cures of most animals (54).

Figure 7 summarizes a study in which Rap-hLL1 specifically targeted Daudi xenografts, and yielded long-term survival in all treated mice. SCID mice were injected intravenously with Daudi cells, and treatment was initiated 1 day after injection of the tumor cells. A single dose of 15 μg of 2L-Rap-hLL1-γ4P yielded cures in 100% of the animals. Treatment with 2 μg rRap (corresponding to the amount in 15 μg 2L-Rap-hLL1-γ4P) or 50 μg of non-specific immunotoxin (2L-Rap-hRS7) had no therapeutic effect. Treatment with 13 μg naked hLL1-γ4P or with a mixture of 13 μg naked hLL1-γ4P and 2 μg rRap led to an approximate doubling of the median survival times observed in mice injected with saline, but was significantly less effective than 2L-Rap-hLL1-γ4P (54).

Immunogenicity, vascular leak syndrome, and nonspecific hepatotoxicity are among major concerns of immunotoxins constructed with plant and bacterial toxins (60, 61). Because the native sequence of Rap lacks the amino acid sequence motif that is responsible for binding to endothelial cells (62), vascular leak syndrome may be less of a concern for Rap-containing immunotoxins. Evaluations of this question as well as potential immunogenicity of 2L-Rap-hLL1-γ4P are required. Toxicity studies of 2L-Rap-hLL1-γ4P in mouse models demonstrated a nonspecific hepatotoxicity of the immunotoxin when elevated doses were administered. This is not unexpected, because the Rap-hIgG fusion proteins are highly basic (p*I* above 10) and earlier work with *Pseudomonas* immunotoxin had demonstrated that increased liver toxicity could be correlated with higher p*I* values. The excellent therapeutic index of 2L-Rap-hLL1-γ4P in xenograft models (about 50-fold above hepatotoxic doses), together with the successful clinical studies using *Pseudomonas* immunotoxin (63), suggest that clinical studies of 2L-Rap-hLL1-γ4P in lymphomas and myelomas are warranted. In addition, the high therapeutic index of 2L-Rap-

hLL1-γ4P recombinant immunotoxins produced as reported by Chang et al. (54) have additional practical advantages, including simple and scalable purification processes, homogeneous and fully functional products, and comparable yields to those of humanized antibodies.

Conclusions

The biological function of CD74 is just beginning to be appreciated in normal and malignant cells, where it appears to serve as a signaling molecule in cell proliferation and survival, as well as having a potential role in innate and adaptive immunity. These functions are being elucidated by the use of CD74 antagonists, including specific antibodies, such as the LL1 antagonistic antibody developed by our group. Preclinical studies of the humanized anti CD74 mAb hLL1 have shown that it is an effective therapeutic agent that may be of significant value for treatment of B-cell malignancies, such as NHL and MM. To the best of our knowledge these studies were the first to suggest that CD74-antagonistic antibodies could be potential therapeutic agents for the treatment of CD74-expressing malignancies. Application of hLL1 should be examined as a single–agent therapy and also as part of multiple-agent therapies. Moreover, hLL1 may be especially useful as a carrier of therapeutic drugs, radioisotopes, or toxins, due to internalization and rapid turnover of the CD74 antigen when bound with this antibody. In light of the expression of CD74 in certain nonhematologic malignancies, the potential of hLL1 as therapy for these diseases also should be evaluated. Development of hLL1 for clinical use is currently in progress.

In addition to the development hLL1 as potential therapeutic, the work described in this review has resulted in observations and methodologies that may be applicable in other systems. Specifically, the approach to the development of recombinant immunotoxins may be applied to the construction of a variety of immunoconjugates containing different toxin moieties and antigen-specific antibodies. In addition, the demonstration that Auger-emitting radioisotopes, such as ^{111}In, and α-particle emitting radionuclides, such as ^{213}Bi, can be used effectively for radioimmunotherapy with an appropriate mAb, have advanced the field of radioimmunotherapy by suggesting that these may be advantageous in the treatment of dispersed, single-cell disease, such as leukemias.

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FIGURE LEGENDS

Figure 1. Immunohistochemical staining of trephine bone marrow biopsy of a MM patient. Section ***A*** is stained with mAb LL1 (anti-CD74), and ***B*** with mAb TAL.1B5 (anti-HLA-DR alpha chain). Immunohistochemistry was performed by indirect immunoperoxidase staining of tissue sections with hematoxylin counter-stain as previously described (22).

Figure 2. hLL1 inhibits proliferation of cell lines. Anti-proliferative effects of the anti-B-cell MAbs were assessed by measuring the uptake of [^{3}H]-thymidine. Cells were cultured with the MAbs with or without a second antibody for crosslinking to mimic the role of effector cells or crosslinking molecules present *in vivo*. Error bars represent standard deviations. ***A.*** NHL cell lines incubated with hLL1; ***B.*** NHL cell lines incubated with rituximab; ***C.*** MM cell lines incubated with hLL1; ***D.*** MM cell lines incubated with rituximab. *Not determined.

Figure 3. Therapy of disseminated MM xenografts in SCID mice with hLL1. MAb hLL1 was administered to MC/CAR-bearing SCID mice at four dose schedules. Survival was compared to untreated MC/CAR-bearing SCID mice. △, 350 μg administered 5 days after tumor cells were grafted; ○, 350 μg/injection once a week for two weeks, treatment initiated 5 days after tumor cells were injected; ▲, 100 μg/injection 5x/week for two weeks, then twice weekly, treatment initiated 5 days after tumor cells were inoculated; □, 100 μg/injection 5x/week for two weeks, then twice weekly, treatment initiated 1 day after tumor cells were injected; and ●, untreated control.

Figure 4. Therapy of disseminated Raji xenografts in SCID mice with ^{111}In-labeled LL1. ***A.*** Five days after i.v. injection of tumor cells, groups of 10 mice were injected with 350 μCi of either ^{111}In-labeled LL1 (△), or ^{111}In-labeled MN-14, an anti-CEA mAb, unreactive with Raji (□). A control group of 10 mice had no antibody injected (○). ***B.*** Groups of 10 mice were treated on day 5 with 275 (▽), 92 (△), or 31 (□) μCi of ^{111}In-labeled LL1. Results with 12 control untreated mice are also shown (○). ***C.*** The time of antibody injection was day 3 (*triangles*), day 5 (*circles*), or day 9 (*squares*) after tumor cell injection, with a dose of 350 μCi of ^{111}In-labeled LL1. Results with untreated control mice (groups of 10-11 mice, *open symbols*) and antibody treated mice (groups of 10 mice, *filled symbols*) are shown.

Figure 5. Dox-hLL1 is cytotoxic on CD74 expressing NHL and MM cell lines. The drug concentration responsible for 50% growth inhibition (IC50) in the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was determined using free-dox, hLL1-dox and hMN-14-dox (irrelevant mAb control). NHL and MM cell lines with various levels of CD74 expression (as measured by indirect flow cytometry assay) were used. Cells were placed in 96-well plates and treated with free dox (*black bars*), hLL1-dox (*gray bars*), or nonreactive conjugate hMN-14-dox (*white bars*) for 4 days. Media was then removed and replaced with media containing MTT, and plates were incubated for 4 h at 37° C. The plates were centrifuged and DMSO added prior to reading in a plate reader at 490 nm.

Figure 6. Therapy of disseminated NHL and MM xenografts in SCID mice with hLL1-dox. SCID mice (8-10 per group) were injected intravenously with 2.5 x 10^6 Raji NHL cells (***A***) or 1 x 10^7 MC/CAR MM cells (***B***). After 5 days, mice were treated with single i.v. doses of dox conjugates of hLL1 (○) and murine LL1 (△), in comparison with control groups including

animals given the same dose of free dox (□), unconjugated hLL1 alone (■), a mixture of free dox + unconjugated hLL1 (▲), untreated (●), and a nonreactive conjugate, hMN-14-dox (□).

Figure 7. Therapy of disseminated Daudi NHL human xenograft with 2L-Rap-hLL1-γP. SCID mice (7 to 8 per group) were injected intravenously with 1.5 x 10^7 Daudi cells. After 1 day, mice were treated with a single bolus i.v. injection of 15 μg 2L-Rap-hLL1-γP (●). Control animals received saline (□), 13 μg naked hLL1 (○), 2 μg rRap (◇), a mixture of naked hLL1 and rRap (△), and a non specific immunotoxin 2L-Rap-hRS7 (□).

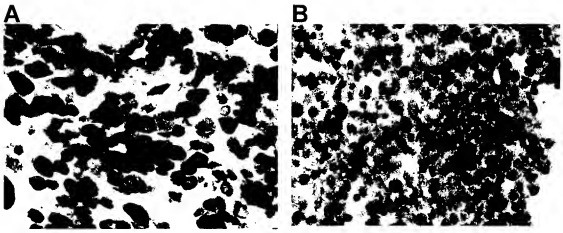
Table 1. LL1 Immunohistology of Patient Biopsy Specimens

Diagnosis	# Positive/# Tested	% Target cells stained
Follicular lymphoma	8/9	>95%
Diffuse large B-cell lymphoma	4/4	~80%
Other NHL	31/35	N.D.
Chronic lymphocytic leukemia/Small lymphocytic lymphoma	14/14	>90%
Multiple myeloma	19/22	16/22 >95%, 3/22 ~50%
Waldenström's macroglubulinemia	2/2	30-50%

Table 2. Effects of combining hLL1 with the chimeric anti-CD20 MAb rituximab and the humanized anti-CD20 MAb, IMMU-106, on proliferation of cell lines. ^{3}H-thymidine uptake is shown as a percentage of the uptake in untreated control cells. Results are shown only for incubations performed in the presence of second antibody.

	Relative Proliferation (%)				
	hLL1	**Rituximab**	**Rituximab+hLL1**	**IMMU-106**	**IMMU-106+hLL1**
FSCCL	19.7±6.2	111.2±8.0	12.1±3.9[a]	109.5±0.8	15.6±3.0[b]
SU-DHL-4	60.8±3.6	92.0±1.1	40.3±1.9[a,c]	85.1±1.4	34.6±2.2[b,c]
RL	86.9±4.9	61.2±7.5	62.7±1.7[c]	67.3±6.6	65.9±4.6[c]
DoHH2	32.1±3.7	43.9±4.5	22.8±3.9[a,c]	45.1±5.6	22.6±1.1[b,c]
Daudi	52.2±9.1	21.1±3.0	15.1±0.4[c]	20.0±3.7	23.4±1.6[c]
MC/CAR	23.9±3.2	39.6±3.6	19.3±1.1[a]	60.7±5.9	23.5±3.3[b]

[a]$P<0.05$ compared to rituximab; [b]compared to IMMU-106, [c]compared to hLL1 by Student's t-test.



LL1 (CD74) TAL.1B5 (HLA-DR alpha chain)

Figure 1

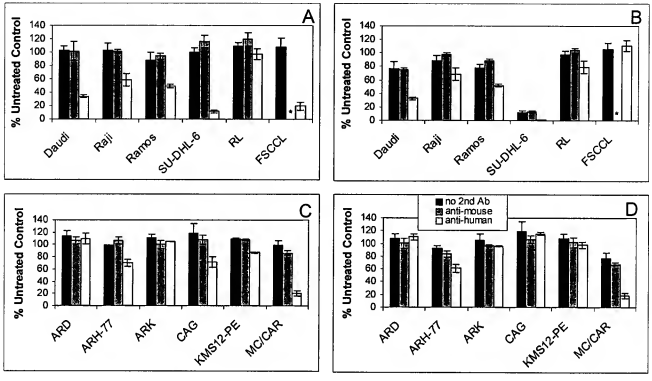

A
B
C
D
% Untreated Control
0
20
40
60
80
100
120
140
Daudi
Raji
Ramos
SU-DHL-6
RL
FSCCL
ARD
ARH-77
ARK
CAG
KMS12-PE
MC/CAR
no 2nd Ab
anti-mouse
anti-human

Figure 2

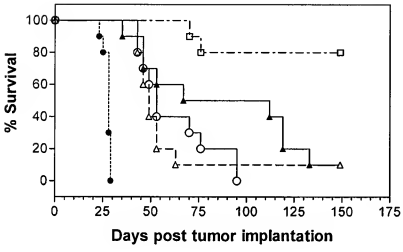

% Survival
100
80
60
40
20
0
0
25
50
75
100
125
150
175
Days post tumor implantation

Figure 3

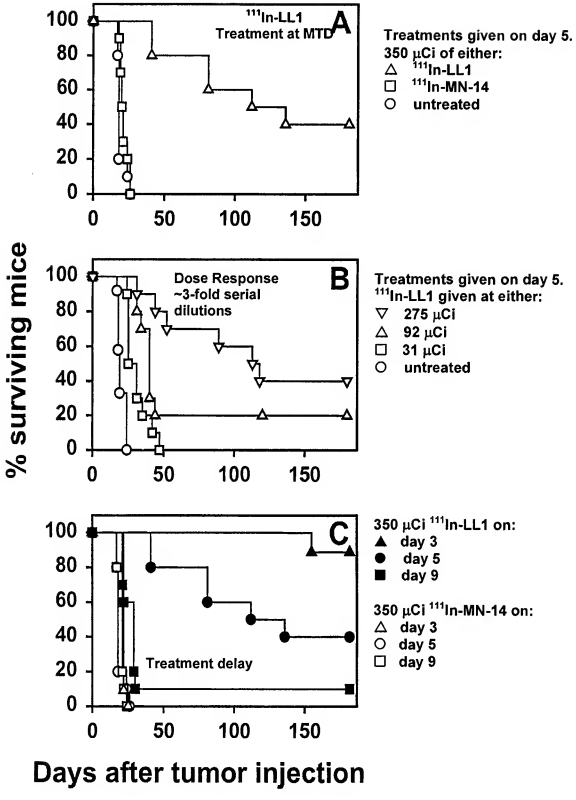
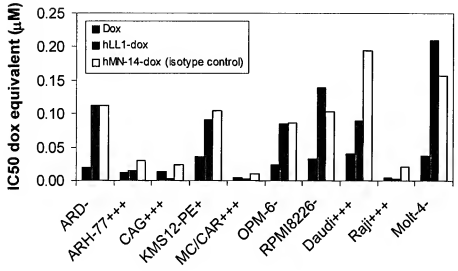

A
^{111}In-LL1
Treatment at MTD
Treatments given on day 5.
350 μCi of either:
△ ^{111}In-LL1
□ ^{111}In-MN-14
○ untreated
B
Dose Response
~3-fold serial
dilutions
Treatments given on day 5.
^{111}In-LL1 given at either:
▽ 275 μCi
△ 92 μCi
□ 31 μCi
○ untreated
C
Treatment delay
350 μCi ^{111}In-LL1 on:
▲ day 3
● day 5
■ day 9
350 μCi ^{111}In-MN-14 on:
△ day 3
○ day 5
□ day 9
% surviving mice
Days after tumor injection
0
20
40
60
80
100
0
50
100
150

Figure 4



CD74 expression: +++, >80% positive +, 10-20% positive -, <10% positive

Figure 5

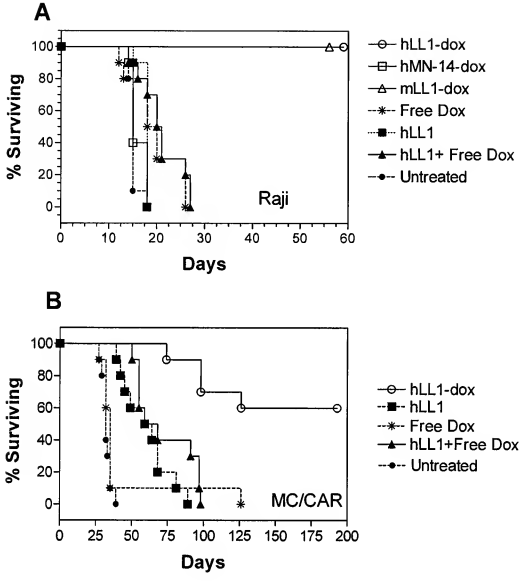

A
% Surviving
Days
Raji
hLL1-dox
hMN-14-dox
mLL1-dox
Free Dox
hLL1
hLL1+ Free Dox
Untreated
B
% Surviving
Days
MC/CAR
hLL1-dox
hLL1
Free Dox
hLL1+Free Dox
Untreated

Figure 6

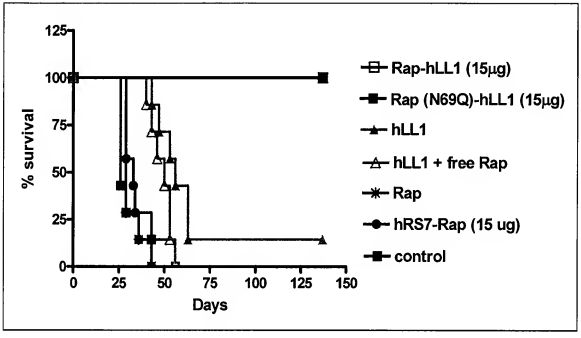

125
100
75
50
25
0
% survival
0
25
50
75
100
125
150
Days
Rap-hLL1 (15μg)
Rap (N69Q)-hLL1 (15μg)
hLL1
hLL1 + free Rap
Rap
hRS7-Rap (15 ug)
control

Figure 7